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Changes in the volatile profile of skim milk powder prepared under different processing conditions and the impact on the volatile flavor profile of model white chocolate

STEWART

In this paper we demonstrate that changes in the processing conditions used to manufacture skim milk powder (SMP) can have an impact on the volatile profile of white chocolate. In particular, we have investigated the roles of heat treatment and the drying process on the development of volatile compounds in SMP. Furthermore, we have investigated how the SMPs manufactured under different conditions behave during a typical conching process in a model white chocolate system. The information presented is of use to both the dairy and the confectionery industries in controlling flavor in their products.

CHANGES IN FLAVOR PROFILE OF SKIM MILK POWDER

Changes in the volatile profile of skim milk powder prepared under different processing conditions and the impact on the volatile flavor profile of model white chocolate

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ABSTRACT

The objective of this work is to determine the extent to which changes in the skim milk powder (SMP) manufacturing process alter the volatile profile of SMP, and whether these changes are carried through to a final product when the SMP is used as an ingredient and subjected to further processing. The manufacture of SMP is a multistage process involving a preliminary concentration step, heat treatment and a drying stage. However, the methods and conditions used by the industry are not standardized, and the inherent variability in the production of SMP has consequences for the end-users, such as the confectionery industry, where the SMP is used as an ingredient during the production of milk chocolate, white chocolate and caramel.

This study investigates the impact of each stage of the manufacturing process on the concentration of reducing sugars and available amino groups (as precursors of the Maillard reaction) as well as on the volatile products of the Maillard reaction and lipid degradation. Eight types of SMP were produced using combinations of different processing conditions: concentration (by evaporation or reverse osmosis), heat treatment (low heat or high heat) and drying (spray-drying or freeze-drying). Maillard precursors were quantified after each processing stage and volatile compounds were extracted using solid-phase microextraction, and analyzed by GC-MS.

The resulting SMPs were incorporated into a model white chocolate system, produced under varying conching conditions. We demonstrate not only that changes in the SMP manufacturing conditions affect the volatile profile of SMP, but also that these differences can be carried through to a final product when the SMP is used to prepare a model white chocolate. Understanding these differences is important to the industry for controlling the flavor of the end product.

53 Key words: manufacture of skim milk powder, flavor, spray dry, freeze dry, chocolate

INTRODUCTION

The manufacture of SMP is a multistage process involving a preliminary concentration step, heat treatment often included to control the functional properties of the final powder (Oldfield et al., 2005), and a drying stage. Since these all involve a rise in temperature, lipid degradation and the Maillard reaction can occur during any of these steps. The severity of the heat treatment applied to milk during milk powder production is classified by industry according to the levels of undenatured whey proteins present i.e. whey protein nitrogen index (WPNI). High heat powder, medium heat powder, and low heat powder have WPNI ranges of < 1.5, 1.5 - 6.0 and > 6.0 mg/g respectively and these can be achieved using a range of different time temperature combinations. Low heat SMP is typically treated at 75 °C for 20 s whereas medium heat conditions range from 85 to 105 °C for 1–2 min, and high heat up to 135 °C for 2–3 min (Early, 1998). Given this range of conditions, the extent of the Maillard reaction in SMP is variable. An understanding of the critical control points during the manufacturing process is important to industries that require a consistent product. The changes in milk powder during storage are well documented (Drake et al., 2006, Driscoll et al., 1985, Hurrell et al., 1983, Karagül-Yüceer et al., 2002, Karagül-Yüceer et al., 2003). Most studies show that the formation of lipid-derived volatiles is prevalent during storage, contributing to the development of off-notes, but both formation and loss of Maillard reaction products were reported, depending on the conditions. Research on high temperature processes in milk tends to focus on UHT (Celestino et al., 1997, Morales et al., 1992, Romero et al., 2001, Tokuşoğlu et al., 2004, Valero et al., 2001) and sterilization (Contarini et al., 1997). The formation of Maillard intermediates and glycation products during manufacture of dairy products has been studied (Birlouez-Aragon et al., 2004, Cattaneo et al., 2008, Erbersdobler and Somoza, 2007), but the focus of these studies was the reduction in nutritional value as a result of lysine residues becoming

unavailable (Mehta and Deeth, 2016). The development of volatile aroma compounds during the production of milk powder was studied by Drake et al., (2006) who showed that Maillard derived compounds such as 2-acetylpyrrole, 2-acetylthiazole and 2-acetyl-2-thiazoline increased, whereas, there was little change in the profile of the lipid degradation products. However, Li et al. (2012) monitored volatile lipid oxidation compounds during the production of milk powder, and demonstrated that all stages of the process could influence the formation and stability of these volatiles.

Recently, the role in flavor formation of the individual unit operations have been investigated. Falling-film evaporators are used extensively in the dairy industry and evaporation under vacuum results in the milk being heated to a lower temperature. Other concentration methods include membrane separation techniques such as reverse osmosis (Glover, 1985), which operates at high pressure and temperatures below those reached during evaporation. Comparison of reverse osmosis, nanofiltration and ultrafiltration was discussed by Syrios et al. (2011) with regard to stability, pH, calcium content and gel formation. Park and Drake (2016) showed that concentration by reverse osmosis, compared to concentration by evaporation, retained far more of the sweet character of the milk, driven by a greater retention of most volatiles, particularly lactones and furaneol. Maltol however, showed the reverse trend. Park et al. (2016) also showed significant changes in the volatile profile when different spray-drying parameters were employed. They showed that the sweet aromatic note increased as the inlet temperature increased, and this correlated with an increase in some lactones, maltol and vanillin.

Given the significant changes in SMP brought about by different processing conditions, it is important to understand if these changes are reflected in the final products when SMP is used as an ingredient for the manufacture of more complex food products. Caudle et al. (2005) showed a decrease in consumer acceptability of SMP as the storage time increased up to 4

years, and when these SMPs were incorporated into ice cream, yogurt and white chocolate (but not hot chocolate) a similar decrease in consumer acceptability was observed. Volatile analysis of the SMP showed an increase in dimethyl sulfide and dimethyl disulfide, and a decrease in maltol. Lloyd et al. (2009b) carried out a similar experiment with stored WMP incorporated in white and dark chocolate and showed a similar decrease in consumer acceptance which was attributed to an increase in lipid degradation products. Recently, Stewart et al. (2017) showed that the heat treatment applied during SMP manufacture leads to both changes in the aroma profile of the SMP, and flavor changes in white chocolate prepared from the resulting SMPs.

The aim of this work was to investigate different processing conditions during the production of SMP to determine the key stages for flavor development, and to determine whether these changes are carried through to a final product. Eight types of skim milk powders were produced using combinations of different processing conditions. Maillard precursors (sugars and amino acids) and aroma compounds were quantified after each stage. The SMPs were incorporated into a model white chocolate system and heated to mimic conching to determine the impact of milk processing methods on the flavor profile of a final confectionery product.

MATERIALS AND METHODS

Chemicals

Trehalose, glucose, galactose, lactose and lactulose, L-leucine, sodium hydroxide (50% solution in water; 1.515 g/mL), sodium dodecyl sulfate (SDS), ethanol, o-phthalaldehyde (OPA), 2-mercaptoethanol, sodium tetraborate buffer solution (pH 9), 1,2-dichlorobenzene, methanol, all aroma chemical, alkanes C₅ – C₃₀ and diethyl ether were obtained from Sigma-Aldrich Co. (Dorset, UK). The EZ:Faast amino acid analysis kit was purchased from Phenomenex (Macclesfield, UK).

Preparation of Milk Powders

Raw whole bovine milk (RWM_{RO}) (40 kg) supplied by The University of Reading CEDAR Dairy Farm (CEDAR, Reading, UK) was pasteurized at 72 °C for 15 s and separated using a disc bowl centrifuge. The skimmed pasteurized milk (PM_{RO}) was then concentrated to 20% total solids using reverse osmosis (RO) to produce concentrated milk (CM_{RO}). Half of the concentrated milk was then subjected to a heat treatment stage to give a heated concentrated milk (HCM_{RO}), and no heat treatment was applied to the other half. The concentrated milks was then spray-dried (SD) or freeze dried (FD) to produce the following milk powders (MP): SDMP_{RO}, HSDMP_{RO}, HFDMP_{RO}, FDMP_{RO} and HFDMP_{RO}. A second batch of raw whole milk was obtained one week later from the same herd, and the process was repeated concentrating to 20% solids using evaporation (EV) to produce a second set of milks (RWM_{EV}, PM_{EV}, CM_{EV}, HCM_{EV}) and milk powders (SDMP_{EV}, HSDMP_{EV}, HFDMP_{EV}, FDMP_{EV} and HFDMP_{EV}).

Reverse Osmosis. Reverse osmosis was carried out at 60 bar outlet pressure using the RO module described previously by Syrios et al. (2011) until the total solids content was 20%, assessed using a Lactoscope (Quadrachem Laboratories Ltd, London, UK). Changes in the protein, fat, lactose and total solid content were previously measured throughout the process (Stewart et al., 2017). The temperature of concentrated milk during RO was 30 °C.

Evaporation. Evaporation was carried out using a single stage rising film evaporator (pressure = 1.8 bar) until a concentration of 20 % total solids was achieved. Milk was concentrated in 10 kg batches and the temperature of the milk during EV was 54–55 °C.

Heat treatment. Half the concentrated milk was subjected to an additional heat treatment of 5 min at 125 °C, achieved by transferring milk to Duran bottles (80 mL) and heating batches of 7 bottles in an autoclave (CertoClav Steriliser, Traun, Austria). No additional heat treatment was applied to the second half of the concentrated milk.

Spray-drying. Spray-drying was carried out using a NIRO spray dryer (Copenhagen, Denmark) with an A/S NIRO atomizer. The inlet air temperature was fixed at 200 °C and the feed flow rate adjusted to give an outlet air temperature of 80–90 °C. The wet bulb temperature during spray drying was 45–50 °C.

Freeze-drying. Prior to freeze-drying, the milk was frozen at -80 °C for 24 h. Freeze-drying was carried out using a Christ Gamma 2-16 LSC freeze-dryer (120 h, pressure < 0.1 mbar) (Martin Christ, Osterode, Germany).

Preparation of Model White Chocolate

White chocolate was prepared as described by Stewart et al. (2017). The conching process of white chocolate was mimicked using a 250 mL continuously stirred reactor vessel (Atlas Potassium, Syrris Inc., Royston, UK) under different heating conditions. A preliminary study was carried out to test different conching conditions, heating the model white chocolate (produced with commercial SMP) at four temperature/time combinations: 4 h at 50 °C, 4 h at 80 °C, 8 h at 50 °C and 8 h at 80 °C. The two extremes were chosen for a comparison to identify potential differences as a result of conching time and temperature. For each milk powder, two model white chocolates were produced using either mild conching conditions (4 h at 50 °C) or harsh conditions (8 h at 80 °C). Model white chocolate was refrigerated and stored at 4±1 °C prior to analysis. A control white chocolate was also produced containing all ingredients apart from SMP, to confirm that differences observed were as a result of the SMP and not due to other ingredients. This control was conched for 8 h at 80 °C and analyzed under the same conditions as other samples.

Analytical Methods

Prior to analysis, all milk samples were diluted to 8 % solids in water and powder samples were reconstituted in water to 8 % total solids.

Determination of Sugars by Ion Chromatography. An aliquot (400 μ L) of each sample was transferred to an Amicon 0.5 mL 3 kDa MWCO filter (Millipore, Watford, UK) and centrifuged for 20 min at $12,000 \times g$. The filtrate was diluted 200-fold in water and 500 μ L of this diluted sample was combined with 500 μ L of a 40 g/L trehalose solution. Extracts were analyzed using a Dionex ion chromatography system (Dionex Corp., Sunnyvale, USA), which consisted of an AS50 autosampler, LC25 column oven, GS50 pumps, and an ED50 pulsed amperometric detector, running in internal amperometric mode. Separation was carried out on a Carbopac PA1 column (Dionex Corp., Sunnyvale, USA) (250 x 4 mm i.d.) coupled with a guard column (50 mm x 4 mm i.d.), using an injection volume of 20 μ L. A gradient program was set up using water and 200 mM NaOH at a flow rate of 1 ml/min as follows: 40 min at 12 mM NaOH, 5 min at 200 mM NaOH, and finally re-equilibrated for 5 min at 12 mM NaOH. The waveform of the pulsed amperometric detector was as follows: 400 ms at 0.1 V, 20 ms at -2.0 V, 10 ms at 0.6 V, and 60 ms at -0.15 V. Standards of glucose, galactose, lactose and lactulose were used to produce a series of calibration curves, using trehalose as an internal standard, for quantification. Chromeleon software was used to operate the system, as well as for data quantification. All samples were analyzed in triplicate.

Determination of Total Available Amino Groups. Aqueous samples were diluted 5-fold in water, followed by derivatization using OPA and spectrophotometric analysis as described previously by (Brands and Van Boekel, 2001). A calibration curve was obtained from L-leucine, which had been derivatized using the same method. All samples were analyzed in triplicate.

Determination of Free Amino Acids by GC-MS. An aliquot of each aqueous sample (100 μ L) was derivatized using the EZ:Faast free amino acid analysis kit for GC-MS (Phenomenex, Macclesfield, UK). GC-MS analysis was carried out as described previously by Elmore et al.(Elmore et al., 2007).

Measurement of Moisture Content and Water Activity (a_w). Moisture content of the skim milk powders was determined by Karl Fischer titration using an Orion AF8 Volumetric Karl Fischer unit (Thermo Scientific, MA, USA). Milk powder samples (0.2 g) were analyzed at room temperature using CombiTitant 5 (Merck, Darmstadt, Germany) as the titrating agent and methanol as the solvent. The solvent was changed at every replicate and analyses were performed at least in duplicate to give less than 0.08 % difference between readings, which were averaged. The water activity of milk powder samples (0.5 g) was measured at 25 °C using a LabMaster- a_w (Novasina, Lachen, Switzerland).

Microscopy. A small amount of each powder was applied to a microscope slide and observed using a stereoscopic microscope (SZ 60, Olympus, Tokyo, Japan) equipped with a lighting (Highlight 3000, Olympus, UK). Photographic images of each sample were acquired with a digital camera and data capture software (VisiCam 5.0, VWR, Belgium).

Solid-Phase Microextraction/GC-MS (SPME/GC-MS). Volatile compounds were extracted from liquid milk, milk powder and white chocolate samples using an automated SPME/GC-MS system (Agilent), equipped with a DVB/CAR/PDMS Stableflex fiber (Supelco, Bellefonte, USA). Samples (4 g) were weighed into 20 ml glass SPME vials and analyzed using the method detailed previously by Stewart et al. (2015). An internal standard (10 μ l of 130.6 μ g/ml 1,2-dichlorobenzene in methanol) was added to aqueous samples. No internal standard was added to model white chocolate samples but instrument sensitivity was checked by running a standard of 1,2-dichlorobenzene (10 μ l, 130.6 μ g/ml in methanol) at regular intervals. All samples were analyzed in triplicate.

Statistical Analysis

One-way analysis of variance (ANOVA) was carried out using XLSTAT Version 2012.4.02 (Addinsoft, Paris, France) and Tukey's honest significant difference (HSD) test was applied to determine which sample means differed significantly at $p = 0.05$.

RESULTS AND DISCUSSION

Milk powder manufacture

The milkpowders were prepared from milk supplied by the University of Reading CEDAR Dairy farm and processed using the equipment available at the University of Reading Food Processing Centre. This allowed us to control the origin and processing of the milk very closely, but, as such, we could not match the manufacturing conditions used in the industry. Although the processing conditions applied to the milk were selected to match industry heating profiles as closely as possible, the heat load on a smaller scale, may be different. A pilot scale can never be fully representative of a full scale industrial process but this paper provides the evidence required to move to industrial trials where validation of the results could take place.

Maillard Precursors

Eight different SMPs were produced using a combinations of techniques, which varied in the level of thermal processing applied. Reverse osmosis and evaporation were used to concentrate the milk, followed by spray-drying or freeze-drying. Half the milk was also subjected to a heat treatment of 5 min at 125 °C between the concentration and drying stages. Precursors (sugars, free amino acids and available amino groups) were quantified after each stage of processing to monitor progress of the Maillard reaction. **Table 1** shows the differences in concentration of Maillard precursors throughout the processing of SMP. It was necessary to use two different batches of raw whole milk (RWM) – one of which was concentrated by evaporation and the other by reverse osmosis. However, the batches were produced < 7 days apart, and analysis of the raw and pasteurized milks showed only minor differences. Batch 2 (EV) contained higher ($p < 0.05$) concentrations of sugars and free amino acids in comparison to the first batch (RO). We compared the general trends within the first batch (i.e. whether there was a relative increase or decrease after each step) with the general

trends in the second batch, but no quantitative conclusions could be made when directly comparing EV and RO samples.

Sugars. Lactose is the most abundant component in SMP, comprising approximately 50% of the dry weight (Martin et al., 2007), and is known to react at elevated temperatures with free amino groups via the Maillard reaction. **Table 1** shows a loss of lactose during the concentration steps for both RO and EV batches of RWM (loss of 150 and 130 mmol per kg dry weight respectively), and both galactose and glucose also showed losses. One potential route for loss of reducing sugars is via reaction with free amino groups to form a Schiff's base. However, the changes in the concentration of the free amino groups were insufficient to account for the loss of lactose, and no isomerisation to lactulose was observed. The impact of heat treatment was more apparent for lactulose, regardless of the concentration method used. Lactulose was present in samples only after the heat treatment stage and its concentration increased further after spray-drying. Lactulose is formed from the Lobry de Bruyn-Alberda van Ekenstein transformation of lactose during heating, and has been used as a marker for heat treatment in milk (Marconi et al., 2004). Additionally, we noticed that lactulose was not formed after spray-drying alone, as it was not detected in either of the spray-dried milk powders (SDMP) with no heat treatment. This suggests it is the high temperature applied during heat treatment (125 °C) that induces the initial stages of lactose isomerisation, which can then continue during further thermal processing (spray-drying) to form lactulose. These results support the previous use of lactulose as a marker of severity of SMP heat treatment. However work by Berg and Van Boekel (1994) showed that degradation of lactulose to galactose, formic acid and C5/6 compounds occurs after extended heating at high temperatures (> 140 °C). The monosaccharides glucose and galactose were both present at low concentrations in RWM. During processing, we observed an overall decrease in glucose of 30% or more from

RWM to the finished milk powder for all combinations of processing. About 12% was lost during pasteurization (both for RO and EV batches) and there was further loss during heat treatment which was greater for HCM_{EV} than HCM_{RO} and for which we have no explanation. The loss of glucose during freeze-drying of the unheated milk (CM) is more than might be expected and is difficult to explain, whereas upon freeze-drying of the heated milk (HCM), there is no significant change. We observed that the most significant decrease in glucose concentration was from RWM_{EV} to HSDMP_{EV}, which represents the powder produced under the most severe combination of processing conditions.

We found that the loss of galactose during pasteurization (13%) (both RO and EV) was similar to that for glucose but, in contrast to glucose, we found a five-fold increase in galactose concentration after heat treatment for both HCM_{EV} and HCM_{RO}. During the Maillard reaction of lactose with an ϵ -amino group, the glucose moiety is the reducing sugar which participates in the first stage of the Maillard reaction. It may remain bound to protein, or further degrade to form volatile flavor compounds (Van Boekel, 1998) during which an intact galactose unit is cleaved off. This explains why there was an increase in galactose concentration after the heat treatment step, but no equivalent increase for glucose. Consistent with this explanation, we also noticed small increases ($p < 0.05$) in galactose but not glucose when the concentrated milks were spray dried, and this change in galactose was less or insignificant when the concentrated milks were freeze-dried. This is consistent with the higher temperatures reached in the spray-drying process compared to the freeze-drying.

Amino groups. The OPA spectrophotometric assay used to quantify available amino groups gives an approximation of the total number of available amino groups, i.e. those not bound to sugars. The most significant loss we observed was for the high heat spray-dried powders, with 48 % (139 mmol NH₂/kg) lost from RWM_{EV} to HSDMP_{EV} and 59 % (186 mmol NH₂/kg) lost from RWM_{RO} to HSDMP_{RO}. These overall losses are of a similar

303 magnitude to the losses of lactose which are 170 and 190 mmol respectively. Lactose binds to
304 amino groups during the Maillard reaction to form the Amadori rearrangement product,
305 making these amino groups unavailable and not quantified by this technique. Therefore it
306 makes sense that the concentration of available amino groups decreased with increased
307 heating.

308 There was little correlation between the level of thermal processing and the total
309 concentration of free amino acids. Although free amino acids are involved in the Maillard
310 reaction at elevated temperatures, there may also be a small degree of proteolysis during
311 processing, releasing free amino acids from the casein or whey protein. Free amino acids are
312 also regenerated by the Maillard reaction during rearrangement of the Amadori
313 rearrangement product to deoxyosones. This would explain the insignificant changes to the
314 total free amino acid concentration.

315 Figure 1 shows variation in the concentration of lysine which demonstrated a significant
316 decrease in concentration during thermal processing. Lysine residues in milk proteins are
317 considered to be the most important for the Maillard reaction in milk powder due to the free
318 and highly reactive ϵ -amino group, but free lysine also has an additional α -amino
319 group(O'Brien, 2003) which can participate. This is supported by the results of this study, as
320 free lysine concentration can be seen to decrease ($p<0.05$) with increased heating, likely to be
321 the result of the Maillard reaction with lactose. There was a decrease in lysine concentration
322 after concentration by both reverse osmosis and evaporation and, although only the decrease
323 from reverse osmosis was statistically significant, the trend was the same for evaporation.
324 However, the decrease from PM to HCM was significant for both concentration methods.

325 There was generally little difference observed between batches concentrated by EV and RO,
326 compared to the bigger changes in Maillard precursors which took place during the heat
327 treatment, and to a lesser extent during spray drying.

Physical Properties of Skim Milk Powders

The moisture content and water activity (a_w) values measured for each powder are shown in Figure 2. It can be seen that FD powders consistently had higher moisture contents and a_w , compared to SD samples. With the exception of HSDMP concentrated by RO, heat-treated samples had a slightly lower moisture content and a_w than the corresponding unheated samples.

Commercial milk powders are produced with a moisture content of 2-4 % (Smit, 2003) to prevent deterioration by bacterial growth or processes such as the Maillard reaction and lipid oxidation, which would also change the flavor profile of the product. Freeze-dried powders in this study had a moisture content of ~ 5-6%, making them more susceptible to deterioration and subsequent flavor formation. The higher moisture content and a_w had a significant impact on flavor formation after incorporation of the powders into a model white chocolate system, which is discussed below.

Substantial differences can be seen in the surface structure of the powders by microscopy (Figure 3.), which agree with previous findings by Miao and Roos (2004). Whereas spray-dried samples were spherical with a smooth surface, freeze-dried powders were irregular in shape with a surface resembling broken-glass. Despite clear differences in surface structure, lactose is highly likely to be in the amorphous state in both powders as water is removed faster than crystallisation can occur during spray-drying, and lactose molecules are unable to move themselves into a crystalline arrangement in a frozen matrix prior to freeze-drying.

Therefore, though the solid state of lactose is unlikely to be different as a result of the different drying methods, the particle size and shape may have influenced the rate of Maillard reaction taking place. In addition, the higher moisture content of the FDMPs are more likely to allow reactants to come into contact with one another. The optimum moisture content for the Maillard reaction in SMP is 7% (Franzen et al., 1990) above which the system is diluted

and reactants are less likely to come into contact. The FDMPs are closer to this optimum moisture content, which is likely to enhance the Maillard reaction in those powders during further processing.

Volatile Compounds in Intermediate Milk Products (RWM, CM, PM, HCM)

The volatile compounds identified in samples by SPME/GC-MS are shown in Table 2 for raw, pasteurized, concentrated and heat-treated samples. (The reconstituted milk powders are shown in Table 3 and discussed below).

Lipid-derived volatiles. Of the 22 volatile compounds identified, 11 were products of lipid oxidation, primarily straight-chain aldehydes (pentanal, hexanal, heptanal, octanal, nonanal and decanal) and methyl ketones (2-heptanone, 2-nonanone, 2-decanone and 2-undecanone). These compounds have all been identified previously in heated milk (Vazquez-Landaverde et al., 2005) and SMP (Bassette and Keeney, 1960, Shimamura and Ukeda, 2012, Walker, 1972), although they are generally considered to contribute off-notes to SMP flavor. Autoxidation of lipids in milk is catalyzed by both light and heat and therefore any processing stage that applies heat and exposes the milk to light would be expected to increase the concentration of lipid oxidation products.

Interestingly, only 2 of these were identified in the volatile profile of RWM whereas 9 were identified in the pasteurized milk (PM). This could be due to the different flavor release properties of the RWM, which contains 4% fat compared to the other samples which had a fat content of <0.1. The higher fat content can decrease the partitioning of the lipophilic aldehydes and ketones into the headspace. It could also be due to the (relatively mild) processing conditions applied during pasteurization (15 s at 72 °C) initiating the oxidative process. Vazquez-Landaverde et al., (2005) compared by SPME/GC-MS the volatile profile of commercial pasteurised milk at 0, 1, 2 and 3% fat content, and found that there was no consistent or significant decrease in the concentration of these volatiles in the headspace as

the fat content increased, suggesting that the lack of these compounds in the headspace of RWM is not due to differences in the flavor release. However, they also reported the presence of these compounds in the raw milk as well as the pasteurised samples, with few significant differences between them, suggesting that they are not formed during pasteurization. However, these observations were made in commercial samples whereas ours have all been prepared from the same two batches of raw milk.

During the subsequent concentration, comparison of RO and EV showed that CM_{RO} contained more aldehydes (particularly hexanal) and acids compared to CM_{EV} , although the differences were not always significant. This is consistent with Park and Drake (2016) who showed that RO retained in general more aldehydes, lactones and acids. During the subsequent heating step, the aldehydes tended to decrease, and we attribute this to their volatile nature, suggesting that they are lost by volatilisation quicker than they are formed. However, this was not the case for 2-heptanone and 2-nonanone, which demonstrated a consistent increase with each additional processing stage and a significant increase after heat treatment. The longer chain ketones (2-decanone and 2-undecanone) and 2-pentylfuran were almost exclusively formed during the heating step. The trends were consistent across the samples regardless of concentration method.

Free fatty acids (FFAs) have been identified as major contributors to the flavor of milk fat by Schieberle et al. (1993) and were detected in all RWM samples. They have also been reported in SMP (Karagül-Yüceer et al., 2001), although our results show that most were removed when the milk was first pasteurised and skimmed, consistent with Drake et al. (2006). Concentration by RO led to an increase in FFA concentration which continued with heat treatment, although levels were much lower than those observed in the starting RWM. Without a double bond, saturated FFAs are less reactive than unsaturated FFAs and as a result are likely to be more heat-stable at lower heating temperatures, such as those applied

during the concentration step. As part of the pasteurization step, the milk was skimmed by centrifugation immediately followed by pasteurization. It is possible that the large decrease in FFA concentration between RWM and PM could be due to the separation process and the removal of FFA with the milk fat. This would result in an initial decrease in concentration followed by an increase as they are formed via lipid oxidation during further processing. The effect of thermal processing is most apparent for volatile compounds formed as a result of thermal degradation and the Maillard reaction. Sulfur compounds and Maillard reaction products (MRPs) were detected only in heat-treated samples and generally in larger amounts for samples concentrated by RO, although the differences between RO and EV were not significant in most cases.

Maillard reaction products. The first stage of the Maillard reaction in milk involves the reaction of lactose with lysine ϵ -amino groups in proteins to form the Amadori rearrangement product (ARP) lactulosyllysine. This ARP can break down via different pathways to give large numbers of volatile flavor compounds. At low pH, dehydration of the ARP leads to the formation of 2-furfural. The presence of 2-furfural in both HCM samples confirms that the Maillard reaction is taking place to a greater degree under the most severe processing conditions (5 min at 125 °C). Two furan derivatives, 2-furfural and 2-furanmethanol, were previously identified in SMP by Shiratsuchi et al. (1994) but were not thought to contribute to the flavor of milk due to their low concentrations and high odor thresholds (2 and 3 mg/kg respectively (Buttery and Ling, 1995)).

In summary, MRPs were only detected in samples that had undergone the heat treatment step and there was no clear differences between flavor formation in samples concentrated by RO or EV.

Considering that the EV batch had a slightly higher concentrations of Maillard precursors (Table 1) and the EV process involved higher temperatures than the RO process (RO = 35 °C,

EV = 55 °C), it is interesting to find that there were only two significant differences in MRPs when comparing HCM_{EV} and HCM_{RO}, and the general trend was for there to be fewer MRPs in HCM_{EV}. Therefore it seems likely that the difference in temperature between the two methods was not sufficient to cause significant differences in flavor compounds formed from the Maillard reaction.

Dimethyl disulfide and dimethyl trisulfide were previously identified as key contributors to the heated flavor of UHT milk by Al-Attabi et al. (2008) and can be formed by the Strecker degradation of methionine during thermal processing. Similarly, 3-methylbutanal and 2-methylbutanal are Strecker aldehydes formed from leucine (Ramshaw and Dunstone, 1969) and isoleucine (Griffith and Hammond, 1989) respectively. Both have previously been identified in raw, pasteurized and UHT milk (Vazquez-Landaverde et al., 2005) as well as in milk powder (Hall et al., 1985, Lloyd et al., 2009a,b).

Volatile Compounds in Skim Milk Powders

Table 3 shows the volatile compounds identified in reconstituted milk powders, and comparison with samples from the early processing stages reveals that the majority of compounds appear in both sample sets and aldehydes and ketones remain the most abundant group of compounds.

Lipid-derived volatiles. The first thing to observe is that the low solids content prior to spray drying (20% compared to 40-50% typically used in industry), may have promoted lipid oxidation as discussed by Park et al. (2016). We observed a significant difference in the concentration of lipid-derived aldehydes in EV powders, with spray-dried powders consistently having a higher concentration than freeze-dried, thus the highest concentration of lipid-derived aldehydes was found in HSDMP_{EV}. This supports previous work by Li et al. (2012), which concluded that heat treatment of milk prior to concentration and spray-drying

results in accelerated formation of aldehydes and ketones. A similar trend was not observed for all RO powders or for other lipid oxidation products, such as methylketones. Comparison of HSDMP_{EV} and HSDMP_{RO} revealed that 11 of the lipid-derived compounds were higher in the powders prepared by EV. This is unlikely to be related to differences between the batches of milk, since the profiles of the lipid-derived volatiles before concentration were very similar (apart from hexanal which was higher in RO). We suggest that the milk is more prone to lipid oxidation during the evaporation stage where they are exposed to light and oxygen as well as mild thermal conditions. Once initiated, the oxidation continues during heating and spray drying. We suggest that RO is likely to be a more effective concentration method to limit the formation of lipid oxidation products during manufacture of SMP, particularly if it is heated and spray-dried. Hexanoic acid was only detected in RO samples and although there was no difference between heat treatments the freeze-dried samples had significantly higher concentrations than their spray-dried equivalents. Conversely, octanoic acid was detected in both EV and RO samples but only the EV samples showed significant differences, as a result of both drying method and heat treatment. Loss of volatile fatty acids during conching was shown to take place by Hoskin and Dimick (1979) and spray-drying of milk powder could yield similar results. The temperature of milk particles during spray-drying (wet bulb temperature) was – 50 °C and in combination with the evaporation of water this could have led to the lower concentration of FFAs in SDMP compared to FDMP produced from the same HCM.

Maillard reaction products. Significant differences were seen between powders for sulfur compounds and MRPs, which were generally at higher concentrations or only present in heated samples, consistent with Drake et al. 2006. Dimethyl trisulfide is one of the compounds which is likely to contribute to the flavor profile of the heated treated powders (Stewart et al., 2017). It was only detected in the heat treated milks and in the powders

produced from the heat treated milks, and there was no difference between the products prepared by EV or RO. The less odor active dimethyl disulfide showed a similar trend. Two Maillard-derived compounds, benzaldehyde and 3-hydroxy-2-methyl-4H-pyran-4-one (maltol), were significantly affected by the combination of concentration, heat treatment and drying methods used. Karagül-Yüceer et al. (2001) previously identified maltol in SMP of various heat treatments, but a higher intensity was perceived in the high heat-treated powder. Maltol is derived from the Maillard reaction of disaccharides such as lactose at high temperatures (Patton, 1950, Yaylayan and Mandeville, 1994) and was only detected after the most severe processing (HSDMP_{EV}). It is one of the compounds reported by Stewart et al., (2017) which are likely to contribute to the more caramel-like flavor in the heat treated powders.

The amount of benzaldehyde detected correlated with the level of thermal processing applied and its concentration was in the following order: HSDMP > HFDMP > SDMP, with none detected in FDMP. Benzaldehyde can be formed from the thermal reaction of lactose with phenylalanine (Ramshaw and Dunstone, 1969), and has been identified previously in stored milk powder (Parks and Patton, 1961). Benzaldehyde was detected in a significantly larger amount in HSDMP concentrated by EV: over 8 times that of the next highest concentration. However, based on the results of Stewart et al., (2017), it is unlikely to contribute to the flavor of the SMP.

Model White Chocolate

To evaluate the effect of different milk powder processing conditions after incorporation into a confectionery product, a series of model white chocolate samples was produced under different conching conditions. For this study, each milk powder was used to produce one batch of model white chocolate conched under normal conditions (4 h at 50 °C) and one batch produced under more extreme heating conditions (8 h at 80 °C). Twenty-five volatile

compounds were monitored by SPME GC-MS. The volatile profile of the products conched at 80 °C are shown in Table 4, but those conched at 50 °C showed very few significant differences between the samples and the full data are not shown. Apart from the short chain acids which tended to show no significant difference across the 8 products, the volatiles fall into two groups according to their overall trends across the 80 °C samples.

Lipid-derived volatiles. The lipid degradation products all followed a pattern similar to that shown for 2-heptanone (Figure 4). This included 2-pentanone, 2-nonanone as well as hexanal, heptanal, octanal and nonanal. Although we cannot account for differences in flavor release, the fact that there was no significant difference across the set of samples conched at 50 °C, suggests that the differences observed at 80 °C are not just a result of different flavour release properties of the samples. The increase in these lipid-derived compounds at 80 °C is consistent with (Counet et al., 2002) who showed an increase in 2-heptanone at high temperature conching. The samples made with freeze-dried SMP showed an increase in lipid-derived compounds when compared to the spray-dried equivalents, and we attribute that to the increase in moisture content of the freeze dried material. Within the products made with spray-dried SMP, there were no significant differences where different milk processing conditions had been used, consistent with the data from the SMPs.

Maillard reaction products. This comprises a group of Maillard sugar breakdown products: 2-furfural, 2-furanmethanol, methyl 2-furoate and maltol, but interestingly not the pyrazines and Strecker aldehydes which generally require high temperature processing for their formation. These sugar degradation products all follow the trend shown in Figure 4 for maltol. Again we see the highest concentrations present in those samples containing freeze-dried SMP, and attribute this to the increase in moisture. Franzen et al. (1990) found 7% to be the optimum moisture content for the Maillard reaction in SMP. The milk powders produced in this study had moisture contents in the range of 2.7 – 6.1 % (a_w 0.15 – 0.25), which is

below the optimum, and once incorporated into the fat phase of the white chocolate the overall moisture content would be lower again. Thus the slightly higher moisture content of the freeze-dried SMP (Figure 2) moves the system closer to the browning-critical moisture content, thus promoting the formation of these Maillard-derived compounds. However, we also observe that those samples containing freeze-dried SMP, where the milk had been concentrated by evaporation rather than reverse osmosis, contain 10-20 times more maltol and other sugar degradation products. We suggest that this can only be due the formation of precursors as a result of the mild heat treatment applied during the evaporation stage. This is supported by the loss of sugars and free amino groups at this stage - there being a significant loss of free amino groups in the EV concentrated milk but not the RO concentrated milk (Table 1). However, we observe the effect of the heat treatment applied to the milk in the set of products made from spray-dried SMP. The concentration of maltol is significantly higher when the additional heat treatment was applied, and 2-furfural, 2-furanmethanol, acetic acid and hydroxypropanone all followed a similar trend. This is consistent with the differences found in the constituent SMPs (Table 3).

CONCLUSIONS

Overall, this work highlights the importance of the combinations and interactions of the model processes chosen for each stage of SMP and confectionery manufacture, on the development of volatile aroma compounds in model white chocolate. Use of evaporation as a drying method seemed to initiate lipid oxidation, which resulted in significantly higher lipid-derived volatiles in the milk powder when further heat treatment was applied. For production of Maillard-derived compounds in SMP, heat treatment was the most important processing stage, and was the first stage at which MRPs were identified. The application of further heat during spray-drying led to increased levels of MRPs compared to

freeze-dried samples. The concentration method seemed to have very little influence on the Maillard reaction, except that maltol, an important aroma compound, was only found in the high heat, spray-dried powder that had been concentrated using evaporation. The volatile profile of the white chocolate was shown to be driven by a number of factors which include moisture content of the SMP, method of concentration and the application of heat. The interrelation of these mechanism is complex, but here we show that changes in these unit operations can quite significantly alter the volatile profile, in particular the combination of concentration by evaporation when the moisture content of the SMP is somewhat higher than is typically used within the industry. In general, we have shown that these stages of processing are interdependent, and early stages of SMP manufacture can have an impact of the volatile profile of model white chocolate. The results of this investigation are potentially useful for the dairy and confectionery industries in controlling the volatile profile of their final product.

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 710

Table 1. Concentration of sugars, free amino acids and available amino groups in samples after each stage of milk powder production (per kg dry weight), concentrated by either evaporation or reverse osmosis

| code | sample | sugars (mmol/kg dry wt.) ^a | | | | available amino groups (mmol NH ₂ /kg dry wt.) ^b | total free amino acids (mmol/kg dry wt.) ^c |
|---------------------|---------------------------------------|---------------------------------------|--------------------------|-------------------------|-----------------------|--|---|
| | | galactose | glucose | lactose | lactulose | | |
| Reverse osmosis | | | | | | | |
| RWM _{RO} | Raw whole milk | 3.6 ± 0.04 ^e | 4.8 ± 0.05 ⁱ | 940 ± 19 ^h | nd ^a | 314 ± 6.7 ^a | 6.76 ^{cd} |
| PM _{RO} | Pasteurized milk | 3.1 ± 0.08 ^d | 4.2 ± 0.07 ^g | 950 ± 19 ^{hi} | nd ^a | 304 ± 11 ^{ab} | 6.68 ^{cde} |
| CM _{RO} | Concentrated milk | 2.4 ± 0.12 ^c | 3.1 ± 0.08 ^{cd} | 790 ± 16 ^{cde} | nd ^a | 289 ± 18 ^{abc} | 5.82 ^{fg} |
| FDMP _{RO} | Freeze-dried milk powder | 2.1 ± 0.04 ^b | 1.7 ± 0.14 ^a | 780 ± 16 ^{cd} | nd ^a | 279 ± 15 ^{abc} | 6.14 ^{def} |
| SDMP _{RO} | Spray-dried milk powder | 3.5 ± 0.09 ^e | 3.0 ± 0.21 ^{cd} | 730 ± 15 ^a | nd ^a | 228 ± 13 ^{efg} | 6.01 ^{ef} |
| HCM _{RO} | Heat-treated concentrated milk | 15 ± 0.4 ^j | 2.7 ± 0.08 ^b | 900 ± 18 ^g | 31 ± 0.6 ^c | 142 ± 5.9 ^h | 5.78 ^{fg} |
| HFDMP _{RO} | Heat-treated freeze-dried milk powder | 16.8 ± 0.2 ^k | 2.9 ± 0.14 ^{bc} | 780 ± 16 ^{cd} | 33 ± 0.7 ^d | 135 ± 12 ^h | 5.95 ^f |
| HSDMP _{RO} | Heat-treated spray-dried milk powder | 17.2 ± 0.2 ^l | 3.1 ± 0.14 ^{cd} | 750 ± 15 ^{ab} | 42 ± 0.8 ^f | 128 ± 7.5 ^h | 4.19 ^h |
| Evaporation | | | | | | | |
| RWM _{EV} | Raw whole milk | 4.0 ± 0.1 ^f | 5.2 ± 0.1 ^j | 970 ± 19 ⁱ | nd ^a | 290 ± 7.5 ^{abc} | 7.91 ^a |
| PM _{EV} | Pasteurized milk | 3.5 ± 0.13 ^e | 4.5 ± 0.08 ^h | 1100 ± 21 ^j | nd ^a | 313 ± 22 ^a | 7.69 ^{ab} |
| CM _{EV} | Concentrated milk | 1.8 ± 0.09 ^a | 4.6 ± 0.07 ^h | 840 ± 17 ^f | nd ^a | 260 ± 3 ^{cde} | 5.20 ^g |
| FDMP _{EV} | Freeze-dried milk powder | 2.0 ± 0.07 ^{ab} | 3.3 ± 0.11 ^e | 820 ± 16 ^{ef} | nd ^a | 273 ± 6 ^{bcd} | 6.22 ^{def} |
| SDMP _{EV} | Spray-dried milk powder | 2.9 ± 0.08 ^d | 3.7 ± 0.08 ^f | 830 ± 17 ^f | nd ^a | 237 ± 22 ^{def} | 6.70 ^{cd} |
| HCM _{EV} | Heat-treated concentrated milk | 8.7 ± 0.23 ^g | 3.1 ± 0.18 ^{de} | 760 ± 15 ^{bc} | 22 ± 0.4 ^b | 194 ± 8.1 ^g | 7.13 ^{bc} |
| HFDMP _{EV} | Heat-treated freeze-dried milk powder | 9.8 ± 0.12 ^h | 3.0 ± 0.04 ^{cd} | 830 ± 17 ^f | 22 ± 0.5 ^b | 203 ± 10 ^{fg} | 5.64 ^{fg} |
| HSDMP _{EV} | Heat-treated spray-dried milk powder | 12 ± 0.1 ⁱ | 1.8 ± 0.08 ^a | 800 ± 16 ^{de} | 35 ± 0.7 ^e | 151 ± 11 ^h | 5.57 ^{fg} |

All samples (except RWM) were adjusted or reconstituted to 8 % total solids content prior to analysis. The total solids content of the raw whole milk was 12%. Results are the mean of three replicate analyses \pm standard deviation. nd = not detected. Means in same column that contain none of the same letters are significantly different ($p = 0.05$)

^a Quantification using a Dionex ion chromatography system without derivatization

^b Derivatization using the EZ:Faast free amino acid analysis kit followed by quantification by GC-MS

^c Derivatization using OPA assay and spectrophotometric analysis

Table 2 Volatile compounds (SPME-GC/MS) in raw whole milk (RWM), pasteurized milk (PM), concentrated milk (CM) and heated concentrated milk (HCM) concentrated using reverse osmosis or evaporation

| LRI ^a | Compound ^b | Relative concentration (µg/L) ^c | | | | | | | |
|----------------------------|-----------------------|--|--------------------------|--------------------------|--------------------------|-------------------------|-------------------------|-------------------------|--------------------------|
| | | Reverse Osmosis | | | | Evaporation | | | |
| | | RWM _{RO} | PM _{RO} | CM _{RO} | HCM _{RO} | RWM _{EV} | PM _{EV} | CM _{EV} | HCM _{EV} |
| Lipid oxidation products | | | | | | | | | |
| 697 | pentanal | nd ^b | 3.1 ± 0.95 ^{ab} | 1.6 ± 1.1 ^{ab} | 4.1 ± 2.4 ^a | nd ^b | nd ^b | nd ^b | 2.6 ± 1.5 ^{ab} |
| 801 | hexanal | 54 ± 1.6 ^b | 330 ± 80 ^a | 94 ± 60 ^b | 59 ± 31 ^b | 11 ± 0.26 ^b | 49 ± 19 ^b | 5.2 ± 2.7 ^b | 4.5 ± 2.9 ^b |
| 901 | heptanal | nd ^c | 40 ± 11 ^a | 24 ± 7.8 ^{ab} | 27 ± 6.3 ^{ab} | nd ^c | 39 ± 15 ^a | 6.3 ± 3.4 ^b | 7.1 ± 4.5 ^b |
| 1002 | octanal | nd ^c | 18 ± 5.2 ^a | 6.1 ± 4 ^{bc} | 9.7 ± 5 ^{abc} | nd ^c | 16 ± 6.1 ^{ab} | 2.8 ± 1.4 ^c | 7.2 ± 4.8 ^{abc} |
| 1103 | nonanal | nd ^b | 52 ± 15 ^a | 17 ± 10 ^{ab} | 17 ± 8.6 ^{ab} | nd ^b | 18 ± 29 ^{ab} | 11 ± 5.7 ^b | 9.8 ± 7.2 ^b |
| 1205 | decanal | nd ^a | 8 ± 10 ^a | 0.86 ± 0.82 ^a | 2 ± 1.2 ^a | nd ^a | nd ^a | nd ^a | nd ^a |
| 889 | 2-heptanone | 3.4 ± 0.66 ^b | 1.6 ± 0.28 ^b | 4.4 ± 2.9 ^b | 120 ± 56 ^a | 3.3 ± 0.52 ^b | 1.9 ± 0.86 ^b | 5.1 ± 2.8 ^b | 100 ± 66 ^a |
| 1090 | 2-nonanone | nd ^c | 1.4 ± 0.34 ^c | 1.1 ± 0.79 ^c | 75 ± 36 ^a | nd ^c | 3.1 ± 1.8 ^{bc} | 1.2 ± 0.64 ^c | 54 ± 37 ^{ab} |
| 1191 | 2-decanone | nd ^b | nd ^b | nd ^b | 1.5 ± 0.63 ^{ab} | nd ^b | nd ^b | nd ^b | 1.7 ± 1.4 ^a |
| 1293 | 2-undecanone | nd ^b | nd ^b | nd ^b | 19 ± 10 ^a | nd ^b | 2.6 ± 1.7 ^b | nd ^b | 13 ± 9.6 ^{ab} |
| 992 | 2-pentylfuran | nd ^a | nd ^a | nd ^a | 51 ± 26 ^a | nd ^a | nd ^a | nd ^a | 240 ± 310 ^a |
| Free fatty acids | | | | | | | | | |
| 781 | butanoic acid | 26 ± 3.7 ^a | nd ^c | nd ^c | 0.42 ± 0.72 ^c | 40 ± 1.7 ^b | nd ^c | nd ^c | nd ^c |
| 969 | hexanoic acid | 98 ± 16 ^b | nd ^c | 4.2 ± 2.4 ^c | 16 ± 12 ^c | 170 ± 22 ^a | nd ^c | nd ^c | nd ^c |
| 1161 | octanoic acid | 63 ± 4.3 ^b | nd ^d | 1.7 ± 1.3 ^d | 29 ± 20 ^c | 87 ± 7.8 ^a | 6.1 ± 1.3 ^d | nd ^d | 3 ± 2.2 ^d |
| Sulfur compounds | | | | | | | | | |
| 748 | dimethyl disulfide | nd ^b | nd ^b | nd ^b | 24 ± 13 ^a | nd ^b | nd ^b | nd ^b | 15 ± 9.3 ^{ab} |
| 978 | dimethyl trisulfide | nd ^b | nd ^b | nd ^b | 58 ± 29 ^a | nd ^b | nd ^b | nd ^b | 25 ± 20 ^{ab} |
| Maillard reaction products | | | | | | | | | |
| 643 | 3-methylbutanal | nd ^b | nd ^b | nd ^b | 3.3 ± 2.4 ^a | nd ^b | nd ^b | nd ^b | 1.5 ± 0.91 ^{ab} |
| 655 | 2-methylbutanal | nd ^b | nd ^b | nd ^b | 3.4 ± 1.6 ^a | nd ^b | nd ^b | nd ^b | nd ^b |
| 835 | 2-furfural | nd ^b | nd ^b | nd ^b | 11 ± 9 ^a | nd ^b | nd ^b | nd ^b | 6 ± 4.2 ^{ab} |
| 854 | 2-furanmethanol | nd ^b | nd ^b | nd ^b | 120 ± 59 ^a | nd ^b | nd ^b | nd ^b | 58 ± 31 ^b |

| | | | | | | | | | |
|-----|--------------|-----------------|-----------------|-----------------|----------------------|-----------------|-----------------|-----------------|----------------------|
| 965 | benzaldehyde | nd ^b | nd ^b | nd ^b | 28 ± 12 ^a | nd ^b | nd ^b | nd ^b | 20 ± 15 ^a |
|-----|--------------|-----------------|-----------------|-----------------|----------------------|-----------------|-----------------|-----------------|----------------------|

^a Linear retention index on DB-5 column, calculated from a linear equation between each pair of straight chain alkanes C₅-C₂₅

^b Compounds identified by comparing the LRI value and mass spectral data with a reference collection (NIST 08)

^c Relative concentration = peak area of compound x concentration of internal standard (ISTD) / peak area of ISTD. Internal standard: 10 µl of 130.6 µg/ml in methanol, nd: not detected. Means of triplicate analyses ± standard deviation, means within the same row not labelled with the same letters are significantly different (p = 0.05)

Table 3 Volatile compounds (SPME-GC/MS) in heated/unheated spray-dried milk powder (HSDMP/SDMP) or freeze-dried milk powder (HFDMP/FDMP) made from milk concentrated by reverse osmosis or evaporation

| LRI ^a | Compound ^b | Relative concentration (µg/L) ^c | | | | | | | |
|----------------------------|-----------------------|--|--------------------------|---------------------------|---------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| | | Reverse Osmosis | | | | Evaporation | | | |
| | | FDMP _{RO} | SDMP _{RO} | HFDMP _{RO} | HSDMP _{RO} | FDMP _{EV} | SDMP _{EV} | HFDMP _{EV} | HSDMP _{EV} |
| Lipid oxidation products | | | | | | | | | |
| 697 | pentanal | 1 ± 0.37 ^{bc} | 0.9 ± 0.4 ^{bc} | nd ^c | nd ^c | nd ^c | 1.5 ± 0.29 ^b | nd ^c | 5.2 ± 1.3 ^a |
| 801 | hexanal | 65 ± 16 ^a | 37 ± 13 ^b | 14 ± 4.4 ^c | 7.6 ± 6.9 ^c | 1.4 ± 0.39 ^c | 11 ± 2.6 ^c | 1.6 ± 0.42 ^{bc} | 37 ± 7.6 ^b |
| 901 | heptanal | 16 ± 11 ^b | 15 ± 5.1 ^b | nd ^b | nd ^b | 2.7 ± 0.73 ^b | 25 ± 5.6 ^b | 6.2 ± 1.3 ^b | 130 ± 28 ^a |
| 1002 | octanal | 4.1 ± 2.5 ^b | 4.1 ± 0.92 ^b | 4.9 ± 1.3 ^b | 7.8 ± 0.34 ^b | 2.8 ± 2 ^b | 14 ± 3.5 ^b | 6.5 ± 1.5 ^b | 100 ± 24 ^a |
| 1103 | nonanal | 15 ± 8.6 ^b | 32 ± 5.4 ^b | 14 ± 3.5 ^b | 44 ± 1.3 ^b | 8.9 ± 1.3 ^b | 44 ± 12 ^b | 17 ± 4.2 ^b | 120 ± 32 ^a |
| 1205 | decanal | 2.2 ± 1.8 ^b | nd ^b | nd ^b | 0.99 ± 0.047 ^b | nd ^b | 2.8 ± 0.8 ^b | 0.66 ± 0.24 ^b | 10 ± 2.8 ^a |
| 889 | 2-heptanone | 2.1 ± 1 ^b | 3.5 ± 2.7 ^b | 9.1 ± 4.2 ^b | 3.2 ± 0.31 ^b | 1.6 ± 0.64 ^b | 6.6 ± 1.7 ^b | 43 ± 12 ^a | 13 ± 3 ^b |
| 1090 | 2-nonanone | 2.4 ± 1.7 ^d | nd ^d | 17 ± 4.8 ^{bc} | 8.1 ± 0.8 ^{cd} | 2.4 ± 3.2 ^d | 5.7 ± 1.8 ^d | 33 ± 7.8 ^a | 21 ± 5.2 ^b |
| 1191 | 2-decanone | nd ^c | nd ^c | nd ^c | nd ^c | nd ^c | 0.77 ± 0.33 ^b | 0.98 ± 0.31 ^b | 1.9 ± 0.42 ^a |
| 1293 | 2-undecanone | nd ^c | nd ^c | 7.9 ± 3 ^{ab} | 4.2 ± 0.5 ^{bc} | nd ^c | nd ^c | 9.3 ± 2.2 ^a | 10 ± 2.8 ^a |
| 992 | 2-pentylfuran | nd ^b | nd ^b | 21 ± 5.8 ^b | 13 ± 2 ^b | nd ^b | 12 ± 2.5 ^b | 44 ± 11 ^a | 60 ± 18 ^a |
| Free fatty acids | | | | | | | | | |
| 969 | hexanoic acid | 7.3 ± 2.8 ^{ab} | 2 ± 0.77 ^{bc} | 7.8 ± 4 ^a | 3.3 ± 2.4 ^{abc} | nd ^c | nd ^c | nd ^c | nd ^c |
| 1161 | octanoic acid | 1.2 ± 0.67 ^c | 0.29 ± 0.26 ^c | 1.7 ± 0.92 ^c | 0.91 ± 0.41 ^c | nd ^c | 5.1 ± 2 ^b | 0.77 ± 0.41 ^c | 15 ± 1.7 ^a |
| Sulfur compounds | | | | | | | | | |
| 748 | dimethyl disulfide | nd ^b | nd ^b | 6.8 ± 1.6 ^{ab} | 13 ± 6.4 ^a | nd ^b | 1.5 ± 0.42 ^b | 5.5 ± 1.8 ^b | 6.4 ± 0.23 ^{ab} |
| 978 | dimethyl trisulfide | nd ^b | nd ^b | 16 ± 2.8 ^a | 27 ± 9 ^a | nd ^b | nd ^b | 16 ± 4.9 ^a | 25 ± 4.2 ^a |
| Maillard reaction products | | | | | | | | | |
| 643 | 3-methylbutanal | nd ^c | 1.4 ± 0.37 ^b | 0.93 ± 0.78 ^{bc} | 5.1 ± 0.59 ^a | nd ^c | 1.6 ± 0.41 ^b | nd ^c | 1.5 ± 0.26 ^b |
| 655 | 2-methylbutanal | nd ^d | 2.2 ± 0.51 ^c | nd ^d | 8.5 ± 1.1 ^a | nd ^d | 2.4 ± 0.7 ^{bc} | nd ^d | 3.7 ± 0.54 ^b |
| 835 | 2-furfural | nd ^d | nd ^d | 2.6 ± 1.8 ^c | 5.6 ± 1.1 ^b | nd ^d | nd ^d | 2.5 ± 0.76 ^c | 16 ± 0.57 ^a |
| 854 | 2-furanmethanol | nd ^b | nd ^b | 20 ± 13 ^{ab} | 21 ± 6.7 ^{ab} | nd ^b | nd ^b | 38 ± 18 ^a | 15 ± 4.4 ^b |
| 965 | benzaldehyde | nd ^b | 1.7 ± 1 ^b | 8.8 ± 0.99 ^b | 11 ± 2.5 ^b | nd ^b | 11 ± 2.7 ^b | 11 ± 3.5 ^b | 94 ± 21 ^a |
| 1116 | maltol | nd ^b | nd ^b | nd ^b | nd ^b | nd ^b | nd ^b | nd ^b | 1.8 ± 0.52 ^a |

^a Linear retention index on DB-5 column, calculated from a linear equation between each pair of straight chain alkanes C₅-C₂₅

^b Compounds identified by comparing the LRI value and mass spectral data with authentic samples

^c Relative concentration = peak area of compound x concentration of internal standard (ISTD) / peak area of ISTD. Internal standard: 10 µl of 130.6 µg/ml in methanol, nd: not detected. Means of triplicate analyses ± standard deviation, means within the same row not labelled with the same letters are significantly different (p = 0.05)

Table .4 Volatile compounds (SPME-GC/MS) in model white chocolate produced from skimmed milk powders produced using different processing conditions, conched for 8 h at 80 °C

| LRI ^a | compound ^b | peak area (x10 ⁵) ^c | | | | | | | |
|------------------|-----------------------|--|----------------------------|---------------------------|---------------------------|---------------------------|--------------------------|-------------------------|---------------------------|
| | | Reverse Osmosis | | | | Evaporation | | | |
| | | FDMP | SDMP | HFDMP | HSDMP | FDMP | SDMP | HFDMP | HSDMP |
| <600 | acetic acid | 140 ± 0.25 ^{de} | 34 ± 1 ^{ab} | 150 ± 3.1 ^e | 74 ± 8 ^{bc} | 260 ± 25 ^f | 58 ± 2.8 ^{abc} | 310 ± 6 ^g | 150 ± 23 ^e |
| 642 | 3-methylbutanal | 6.8 ± 0.68 ^{cd} | 4.3 ± 0.48 ^{abc} | 6.3 ± 0.29 ^{bcd} | 4.2 ± 0.63 ^{abc} | 4.6 ± 0.59 ^{abc} | 4.6 ± 1.7 ^{abc} | 4 ± 1 ^{ab} | 3 ± 0.037 ^a |
| 649 | 1-hydroxy-2-propanone | 13 ± 1.1 ^{ab} | 2.2 ± 0.073 ^a | 35 ± 2.9 ^c | 18 ± 4.8 ^b | 53 ± 3 ^d | 4.4 ± 1.9 ^a | 71 ± 3.3 ^e | 11 ± 1.3 ^{ab} |
| 654 | 2-methylbutanal | 12 ± 0.65 ^a | 5.1 ± 0.63 ^a | nd | 11 ± 0 ^a | nd | 6.1 ± 2.2 ^a | nd | 6.8 ± 1.4 ^a |
| 677 | propanoic acid | 11 ± 4.6 ^a | 7.5 ± 3.3 ^a | 14 ± 3.8 ^a | 10 ± 6 ^a | 9 ± 1.2 ^a | 8.8 ± 5.8 ^a | 7.2 ± 1.6 ^a | 11 ± 0.71 ^a |
| 684 | 2-pentanone | 31 ± 3.1 ^f | 11 ± 0.061 ^{abc} | 24 ± 0.85 ^{def} | 11 ± 1.6 ^{abc} | 26 ± 3.1 ^{ef} | 15 ± 7.1 ^{bcd} | 20 ± 1 ^{cde} | 12 ± 0.73 ^{abc} |
| 698 | pentanal | 360 ± 6.5 ^{abc} | 550 ± 52 ^d | 340 ± 46 ^{abc} | 350 ± 66 ^{abc} | 330 ± 2.3 ^{abc} | 450 ± 85 ^{bcd} | 270 ± 7.5 ^a | 290 ± 40 ^{ab} |
| 782 | butanoic acid | 190 ± 0.59 ^c | 67 ± 7.5 ^a | 120 ± 7.3 ^b | 70 ± 12 ^a | 120 ± 8.2 ^b | 71 ± 9.6 ^a | 110 ± 6.3 ^b | 120 ± 20 ^b |
| 800 | hexanal | 82 ± 3.7 ^{ab} | 65 ± 3.3 ^a | 70 ± 9.3 ^{ab} | 52 ± 10 ^a | 100 ± 2 ^b | 70 ± 17 ^{ab} | 74 ± 8.5 ^{ab} | 51 ± 5 ^a |
| 830 | 3-methylbutanoic acid | 6 ± 0 ^a | 5.4 ± 0 ^a | 5.2 ± 0 ^a | 5 ± 0 ^a | nd | 5 ± 0 ^a | 3.4 ± 0 ^a | 3.5 ± 0 ^a |
| 833 | 2-furfural | 5.1 ± 0.9 ^a | 0.55 ± 0.13 ^a | 3.3 ± 0.31 ^a | 2 ± 0.56 ^a | 60 ± 0.86 ^c | 1.5 ± 0.8 ^a | 26 ± 6.4 ^b | 3.7 ± 0.28 ^a |
| 852 | 2-furanmethanol | 31 ± 1 ^a | 1.6 ± 0.27 ^a | 53 ± 12 ^a | 9.8 ± 1.4 ^a | 780 ± 53 ^c | 3.3 ± 0 ^a | 540 ± 190 ^b | 9.8 ± 2.3 ^a |
| 870 | pentanoic acid | 16 ± 0.31 ^a | 12 ± 4.2 ^a | 13 ± 3.7 ^a | 15 ± 0.51 ^a | 16 ± 0.12 ^a | 20 ± 2.6 ^a | 10 ± 0.17 ^a | 16 ± 4 ^a |
| 889 | 2-heptanone | 88 ± 2 ^{fg} | 41 ± 3 ^{cd} | 67 ± 12 ^{def} | 38 ± 8.2 ^{bcd} | 100 ± 11 ^g | 51 ± 18 ^{de} | 72 ± 13 ^{ef} | 41 ± 4.6 ^{cd} |
| 900 | heptanal | 14 ± 0.2 ^{bc} | 7.8 ± 0.25 ^{ab} | 9.7 ± 0.97 ^{abc} | 6.3 ± 2 ^a | 16 ± 1.7 ^c | 9.2 ± 4.6 ^{abc} | 10 ± 2.5 ^{abc} | 6.8 ± 2.1 ^{ab} |
| 913 | dimethyl sulfone | 95 ± 2.6 ^e | 30 ± 0.76 ^{abc} | 71 ± 6.4 ^{de} | 37 ± 4.8 ^{bc} | 190 ± 4.5 ^g | 46 ± 9.9 ^{cd} | 130 ± 22 ^f | 46 ± 0.52 ^{cd} |
| 956 | (E)-2-heptenal | 5.9 ± 0.71 ^a | 2.4 ± 0.12 ^a | 3.8 ± 1.4 ^a | 3.7 ± 1.3 ^a | 21 ± 6.2 ^b | 7.1 ± 0.75 ^a | 12 ± 9.5 ^{ab} | 4.7 ± 1.1 ^a |
| 965 | benzaldehyde | 3.3 ± 0.56 ^{ab} | 2.1 ± 0.73 ^{ab} | 2.8 ± 1.1 ^{ab} | 1.6 ± 0.13 ^a | 11 ± 0.86 ^c | 3.4 ± 1.3 ^{ab} | 4.6 ± 0.55 ^b | 1.7 ± 0.046 ^{ab} |
| 966 | hexanoic acid | 68 ± 1.7 ^d | 20 ± 4.5 ^{abc} | 37 ± 6.3 ^{bc} | 16 ± 2.6 ^{ab} | 74 ± 3.5 ^d | 21 ± 6.3 ^{abc} | 39 ± 16 ^c | 29 ± 6.2 ^{abc} |
| 1001 | octanal | 11 ± 0.8 ^{bc} | 7 ± 0.34 ^{abc} | 8.9 ± 1.9 ^{abc} | 6.5 ± 1.3 ^{ab} | 19 ± 1 ^d | 8.7 ± 1.4 ^{abc} | 12 ± 3.4 ^c | 7.3 ± 0.96 ^{abc} |
| 1085 | methyl 2-furoate | nd | nd | nd | nd | 26 ± 4.5 ^a | nd | 12 ± 8.4 ^a | nd |
| 1088 | tetramethylpyrazine | nd | 0.64 ± 0.26 ^a | 0.88 ± 0 ^a | 0.56 ± 0.018 ^a | nd | 0.81 ± 0 ^a | nd | 0.71 ± 0.006 ^a |
| 1089 | 2-nonanone | 25 ± 1.8 ^{de} | 8.2 ± 0.068 ^{abc} | 17 ± 4 ^{cd} | 7.8 ± 1.3 ^{abc} | 32 ± 3.1 ^e | 12 ± 2.8 ^{abc} | 16 ± 5 ^{bcd} | 9.1 ± 0.93 ^{abc} |
| 1102 | nonanal | 49 ± 4.9 ^{bc} | 21 ± 1.3 ^{ab} | 35 ± 4.7 ^{ab} | 18 ± 2 ^a | 72 ± 24 ^c | 26 ± 3.7 ^{ab} | 31 ± 9.4 ^{ab} | 18 ± 1.9 ^a |
| 1114 | maltol | 17 ± 0.88 ^a | nd | 15 ± 0 ^a | 2.6 ± 2.2 ^a | 310 ± 3.2 ^c | nd | 120 ± 18 ^b | 1.8 ± 0.13 ^a |

^a Linear retention index on DB-5 column, calculated from a linear equation between each pair of straight chain alkanes C₅-C₂₅^b Compounds identified by comparing the LRI value and mass spectral data with authentic standard^c Peak area from SPME/GC-MS, nd: not detected. Means of triplicate analyses ± standard deviation, means within the same row not labelled with the same letters are significantly different (p = 0.05). FDMP: freeze-dried milk powder, SDMP: spray-dried milk powder, HFDMP: heat-treated freeze-dried milk powder, HSDMP: heat-treated freeze-dried milk powder

FIGURE CAPTIONS

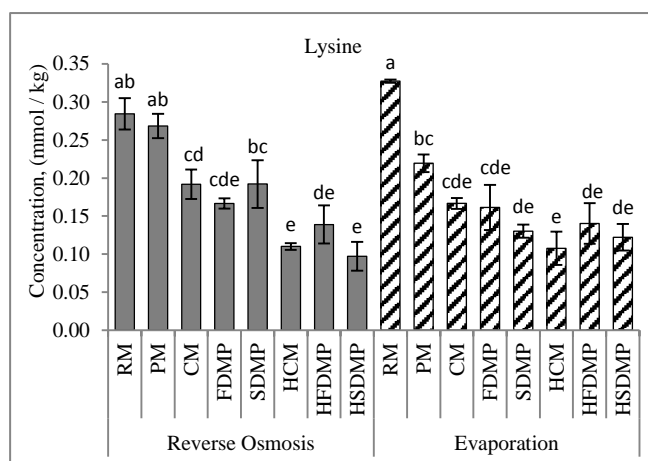
Figure 1. Variation in the concentration of lysine during processing of skim milk powder (SMP) using two different concentration methods: reverse osmosis and evaporation. RWM: raw milk, PM: pasteurized milk, CM: concentrated milk, HCM: heat treated concentrated milk, SDMP: spray-dried milk powder, HSDMP: heat treated spray-dried milk powder, FDMP: freeze-dried milk powder, HFDMP: heat treated freeze-dried milk powder. All samples were made up to 8 % total solids content prior to analysis. Results are the mean of three replicate analyses \pm standard deviation (error bars). Bars not labelled with the same letters are significantly different ($p = 0.05$).

Figure 2. Moisture content (%) of milk powder samples, determined by Karl Fischer titration. Mean of duplicate analyses \pm standard deviation as error bars. Number above each bar denotes the water activity (a_w).

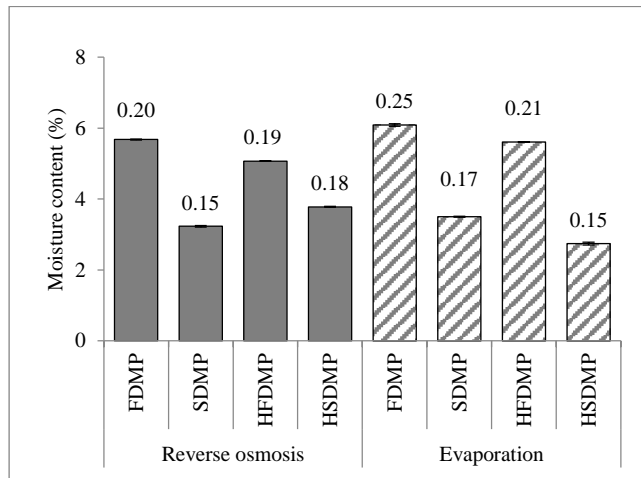
Figure 3. Optical micrographs of heated spray-dried milk powder (HSDMP) or freeze-dried milk powder (HFDMP) produced from milk concentrated by reverse osmosis (RO) or evaporation (EV). (A) HSDMP_{RO}, (B) HSDMP_{EV}, (C) HFDMP_{RO}, and (D) HFDMP_{EV}

Figure 4. Comparative analysis (SPME-GC/MS) of (a) 2-heptanone and, (b) maltol in model white chocolate containing skimmed milk powder produced with different combinations of processing conditions: Concentration by reverse osmosis or evaporation, low or high heat treatment (H: high heat treatment) and drying by either freeze-drying (FD) or spray-drying (SD). Mean of triplicate analyses shown \pm standard deviation as error bars. Bars not labelled with the same letters are significantly different ($p = 0.05$)

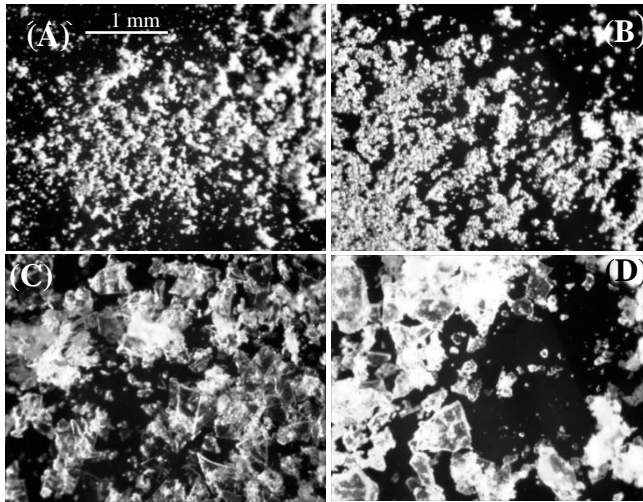
STEWART FIGURE 1



STEWART FIGURE 2



STEWART FIGURE 3



STEWART FIGURE 4

